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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PP5891 for a patent by BIOSCEPTRE PTY LIMITED as filed on 22 June 2001.

I further certify that pursuant to the provisions of Section 38(1) of the Patents Act 1990 a complete specification was filed on 17 January 2002 and it is an associated application to Provisional Application No. PR5891 and has been allocated No. 2002224664.

WITNESS my hand this
Twelfth day of March 2007

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Patents Act 1990
PROVISIONAL SPECIFICATION
FOR A PROVISIONAL PATENT

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Invention Title: **Diagnosis Of Cell Cancers Using An Antibody To Non-Functional P2x7 Receptors**

The following statement is a description of this invention

This invention relates to antibody-based diagnosis of cancers derived from epithelial cells as well as other types including malignant lymphoma.

A basis for the invention is found in research into the purinergic receptor $P2X_7$ in epithelial and other cells. ATP can induce cytolysis in epithelial cells and other cells such as leukocytes including lymphocytes, thymocytes, macrophages and dendritic cells through the $P2X_7$ receptors expressed on the cell surface. $P2X_7$ receptors open channels through the cell membrane within a second. Continued application of ATP leads to the formation of a pore within a few tens of seconds that induces apoptosis. The $P2X_7$ subtype is involved in apoptosis or programmed cell death in many cell types. It is referred to as a cytolytic receptor that forms calcium channels that can be transformed into large pores with continued exposure to adenosine triphosphate (ATP) agonist in order to flood the cell with excess calcium.

In a preferred embodiment, the invention uses a $P2X_7$ subtype-specific antibody to specifically detect non-functional $P2X_7$ receptors expressed within and on epithelial cells forming part of preneoplastic or neoplastic tissue. Thus the receptor is detected in a close-gated or non-functional conformation even though it may be normally expressed in the cell membranes.

It has now been found that, in patients with epithelial cell cancer such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder and vaginal cancer and malignant lymphoma , but not confined to these, non-functional $P2X_7$ receptors can be detected by using an antibody directed against an epitope that undergoes a conformational change from the structure present in functional receptors. It has been found that the amino acid sequence of the non-functional receptors can be identical to the amino acid sequence of functional receptors so that the cause of the conformational change in the receptors relates to interaction of the receptors with ATP. The ATP molecules act as receptor agonists, so that when ATP is bound to the receptors, they are able to open a channel through the cell membrane for the inflow of calcium ions. Non-functionality is therefore caused by a lack of appropriate binding of the ATP agonists to the receptors for reasons that may include a deficit in the local availability of ATP through production deficit or increase in rate of degradation. If ATP binding to the receptors is disrupted, the receptor conformation is altered and this can be detected using an antibody specially designed to bind to the region of the protein affected by the binding of the ATP.

The specific sequence involved in the conformational change includes Pro210 in human $P2X_7$ receptors that undergoes a change in configuration from trans form to cis form in the absence of bound ATP. Thus an appropriate epitope sequence against which an antibody must be raised includes Pro210 and may extend either side of this residue to an appropriate extent necessary to induce an antibody response. This may include by way of example, a segment extending from Gly200-Thr215 but is not confined to this segment.

Because current studies and investigations may not fully explain the working of the invention, it is necessary to define the invention in a number of aspects, as set out below. It is possible and likely that there will be overlap of at least some of those aspects.

Accordingly, in a first aspect, the invention provides an antibody for detection of cancer and preneoplasia, including those cancers derived in epithelial cells listed above and B-cell lymphocytes in malignant lymphoma, the antibody specially adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting change in relation to binding of ATP to the receptors but also allowing for the detection of other regions of the $P2X_7$ receptor unchanged by functional state. The antibody may be polyclonal, monoclonal, recombinant or a humanised antibody or appropriate fragment and is preferably directed against an epitope located in the extracellular domain adjacent to the ATP binding sites and incorporating the proline at amino acid 210 in the human $P2X_7$ sequence that undergoes cis/trans isomerisation, with the cis conformer associated with the non-functional conformation.

It is apparent that the embodiment of the invention covers alternative sequences that similarly distinguish functional and non-functional receptors through detection of the conformational changes occurring when ATP binds so the change detected may be in an amino acid other than the proline referred to above, or in some other respect.

The detection of non-functional $P2X_7$ receptors in these conditions occurs in a pattern in which normal cells remain essentially unlabelled. Thereafter, the non-functional conformation of $P2X_7$ is first detected in the nuclei and cytoplasm of the epithelial cells in preneoplasia and later in neoplasia. This detects preneoplasia up to several years prior to the normal pathological appearance of cancer as detected by haematoxylin and eosin (H&E) stained slides of biopsied tissues such as prostate, skin and fibroadenomas in breast tissue and can thus be used to introduce

early therapy for appropriately selected patients and to monitor the effects of therapy in patients.

In a second aspect the invention describes the use of a novel antibody to human telomerase associated protein 1 or hTP1 able to detect an upregulation of protein in the nucleus and cytoplasm of epithelial cells in the above cancers so that the test for the presence of advanced cancer can be further enhanced by the separate or simultaneous labelling for hTP1 to determine the degree of invasiveness of the cancer.

Diagnosis using the antibody of the invention may be carried out using in situ imaging techniques that may attach a range of probes to the antibody to detect distribution in body tissues as well as standard microscopy, confocal microscopy or fluorescence activated cell sorting as well as normal immunohistochemical techniques of lymph, prostate, breast, skin, lung, uterus, bladder, cervix, stomach, oesophagus and similar biopsies as well as in fine needle aspirates of breast and other tissue and cell smears such as those taken for the detection of cervical cancer but not confined to these.

The invention includes in a third aspect a method for detection of cancer and pre-neoplasia, including those cancers derived in epithelial cells listed above and B-cell lymphocytes in malignant lymphoma, the method including use of the antibody of the invention.

In a fourth aspect, the invention provides a method for detection of upregulation of protein in the nucleus and cytoplasm of epithelial cells in the above cancers when the condition is malignant, the method including the use of the novel antibody to human telomerase associated protein one of hTP1, as defined above.

It will be apparent to those skilled in the art that many obvious modifications and variations may be made to the embodiments described herein without departing from the spirit or the scope of the invention.

Dated this 22nd day of June 2001

Biosceptre Pty Limited

by its Patent Attorneys

Chrysiliou Law